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Ghrelin – a pleiotropic hormone secreted from endocrine X/A-like cells of the stomach

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The gastric X/A-like endocrine cell receives growing attention due to its peptide products with ghrelin being the best characterized. This peptide hormone was identified a decade ago as a stimulator of food intake and to date remains the only known peripherally produced and centrally acting orexigenic hormone. In addition, subsequent studies identified numerous other functions of this peptide including the stimulation of gastrointestinal motility, the maintenance of energy homeostasis and an impact on reproduction. Moreover, ghrelin is also involved in the response to stress and assumed to play a role in coping functions and exert a modulatory action on immune pathways. Our knowledge on the regulation of ghrelin has markedly advanced during the past years by the identification of the ghrelin acylating enzyme, ghrelin-*O*-acyltransferase, and by the description of changes in expression, activation, and release under different metabolic as well as physically and psychologically challenging conditions. However, our insight on regulatory processes of ghrelin at the cellular and subcellular levels is still very limited and warrants further investigation.

Keywords: acyl and desacyl, GOAT, ghrelin, nesfatin-1, NUCB2, endocrine cells, stress

INTRODUCTION

Several enteroendocrine cells have been identified in the stomach and shown to influence physiological functions with a predominant effect on gastric acid secretion, namely gastrin-producing cells (G cells), somatostatin-releasing cells (D cells, 5–10% of gastric oxyntic endocrine cells in rats, >20% in humans), enterochromaffin-like cells releasing histamine (ECL, 65% in rats, 30% in humans), and much less abundantly the serotonin-containing enterochromaffin (EC) cells (Rindi et al., 2004). In addition, a distinct cell type has been identified in the stomach that is distributed throughout the mucosa (**Figure 1**) that was termed X/A-like cell in rats and P/D₁ cell in humans (Date et al., 2000; Mizutani et al., 2009). These cells were named X cells because of their unknown functions and in addition termed A-like cells due to their similarity with pancreatic A-cells (Rindi et al., 2004). They account for 20–30% of the oxyntic endocrine cells and therefore represent the second most abundant gastric endocrine cell type (Rindi et al., 2004). Distribution studies in the rat gastrointestinal tract indicate that the cell density (cells/mm²) of X/A-like cells is 10- to 100-times greater in the gastric body than in the lower intestinal tract (Sakata et al., 2002). At the morphologic level, the X/A-like cells exist as closed-type cells without contact to the lumen and open-type cells with luminal contact. The open-type cells are more prominent in the ileum, cecum, and colon, where they account for more than 60% of ghrelin cells (Sakata et al., 2002). The identification of ghrelin in rat X/A-like and human P/D₁ cells (Rindi et al., 2002) as the only peripherally produced and centrally acting hormone known to increase food intake (Date et al., 2000) dramatically increased the interest in this endocrine

cell type which is now commonly named ghrelin cell (Rindi et al., 2002).

PEPTIDE PRODUCTS OF X/A-LIKE CELLS

Growing interest in ghrelin cells led to the discovery of additional peptide products derived from this cell type. These peptides are either derived from the same ghrelin gene including desacyl ghrelin and *n*-decanoyl ghrelin (Date et al., 2000; Hiejima et al., 2009) as well as obestatin (Zhang et al., 2005) or from a different gene, namely nucleobindin 2 (NUCB2)/nesfatin-1 (Stengel et al., 2009a).

Ghrelin was discovered in 1999 by Kojima and colleagues (reviewed in Kojima and Kangawa, 2011) and identified to be the endogenous ligand of the growth hormone (GH) secretagogue receptor 1a isoform (GHS-R1a; Kojima et al., 1999), which was later renamed ghrelin receptor (GRLN-R; Davenport et al., 2005). Ghrelin is a 28-amino acid peptide which has a unique *n*-octanoic acid residue on the serine-3 thereby increasing its lipophilicity (Kojima et al., 1999) and shown to be essential for binding to the GRLN-R (Kojima et al., 1999; Kojima and Kangawa, 2005). Structure-activity studies established that the first five N-terminal amino acids that include the hydrophobic residue are able to activate the receptor pointing toward the active core of ghrelin (Bednarek et al., 2000). Studies in mice ingesting different concentrations of medium-chain fatty acids (MCFA) or medium-chain triacylglycerols (MCT) established their direct use as a source for ghrelin acyl modification (Nishi et al., 2005). Without this post-translational modification desacyl ghrelin is obtained which does not bind to the GRLN-R. The gastric endocrine X/A-like cells are

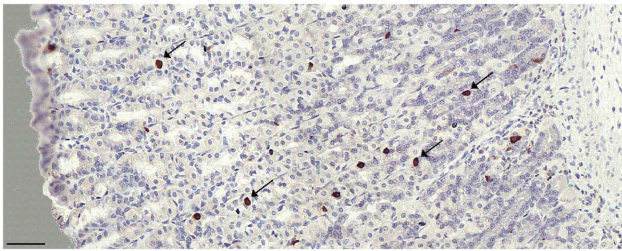


FIGURE 1 | Immunohistochemical photomicrograph of X/A-like cells in the rat gastric oxyntic mucosa of *ad libitum* fed male rats.

Ghrelin-positive X/A-like cells (arrows) are evenly distributed throughout the entire length of the gastric oxyntic glands. The scale bar represents 50 μ m.

the major source of circulating desacyl and acyl ghrelin and the ratio of acyl and total (both acyl and desacyl) ghrelin in the circulation has been initially reported to be between 1:15 (Hosoda et al., 2000) and 1:55 (Raff, 2003). Recent improvements in blood processing resulted in a markedly higher acyl/total ghrelin ratio of 1:5 compared to 1:19 obtained after standard blood processing (EDTA blood on ice; Stengel et al., 2009b) indicating that although desacyl ghrelin represents the major form of circulating ghrelin, previous values were skewed by suboptimal blood processing conditions to preserve acyl ghrelin that is easily cleaved by a wide range of cellular protease and during protein extraction. Recently, another acylated form of ghrelin has been identified in humans and rodents, *n*-decanoyl ghrelin, which is also derived from X/A-like cells and circulates in considerable amounts in the mouse blood (Hiejima et al., 2009).

The enzyme catalyzing the acylation of ghrelin was unknown for a decade and recently identified in mice and humans as a member of the superfamily of membrane-bound *O*-acyltransferases (MBOATs), MBOAT4 that was renamed ghrelin-*O*-acyltransferase (GOAT; Gutierrez et al., 2008; Yang et al., 2008). GOAT is thought to octanoylate proghrelin before being transported to the Golgi apparatus where it is cleaved by prohormone convertase 1/3 (PC 1/3; Yang et al., 2008). Recently, GOAT protein was also identified in rodent circulation (Stengel et al., 2010d) leading to the possibility of an extracellular acylation of ghrelin. Both MCFAs C8 and C10 are substrates for GOAT-catalyzed acylation resulting in octanoyl and decanoyl ghrelin (Gutierrez et al., 2008). A current study reported the development of an antagonist of GOAT, a peptide-based bisubstrate analog GO-CoA-Tat, shown to be a useful tool *in vitro* and *in vivo* to assess the relevance of GOAT in body weight and glucose regulation (Barnett et al., 2010).

Alternative splicing and post-translational modification at a computer-based predicted cleavage site of proghrelin was reported to result in another biologically active peptide which was termed obestatin and assumed to have opposite effects to those of ghrelin (Zhang et al., 2005; Soares and Leite-Moreira, 2008). Obestatin immunoreactivity is also found in human gastric endocrine P/D₁ cells and localized in secretory granules (Gronberg et al., 2008; Tsolakis et al., 2009). Similarly, in rats obestatin fully colocalized with preproghrelin in intracellular dense core granules of gastric

endocrine cells, whereas only partial (60%) colocalization of ghrelin and obestatin have been described giving rise to differential post-translational expression (Zhao et al., 2008).

NUCB2/nesfatin-1 was initially identified in the rat hypothalamus (Oh-I et al., 2006) but recently shown to be also expressed in the gastric oxyntic mucosa, prominently in gastric oxyntic endocrine cells (Stengel et al., 2009a). Colocalization of ghrelin and nesfatin-1 in rat gastric X/A-like cells was identified by immunofluorescence within different pools of vesicles indicative of a distinct subcellular distribution (Stengel et al., 2009a). Coexpression of these two peptides in X/A-like cells is also supported by the presence of PC 1/3 in this cell type (Yang et al., 2008) which is involved in the processing of both, ghrelin and nesfatin-1 (Yang et al., 2008; Shimizu et al., 2009).

Despite the fact that the functions of obestatin remain highly controversial (Goebel et al., 2008) and those of desacyl ghrelin (Chen et al., 2009) and nesfatin-1 (Garcia-Galiano et al., 2010) are just starting to be understood, all peptide products derived from this cell seem to be involved in the regulation of food intake with a stimulatory action of ghrelin and an inhibitory effect of desacyl ghrelin and nesfatin-1 (Stengel et al., 2010c).

REGULATION OF GHRELIN RELEASE AND RECEPTOR INTERACTIONS

Ghrelin-positive X/A-like cells represent by far the major source of circulating ghrelin (Ariyasu et al., 2001) as demonstrated by the sharp decrease of circulating ghrelin following gastrectomy (Jeon et al., 2004). In addition, lower amounts of ghrelin are produced in the intestine (Date et al., 2000), pancreas (Date et al., 2002b) and other peripheral organs including the kidney, liver, heart, testis, adipose tissue, and skin (Barreiro et al., 2002; Gnana-pavan et al., 2002). Circulating ghrelin levels vary with metabolic status rising before and declining after a meal in various experimental animals and humans (Cummings et al., 2001; Tschöp et al., 2001a). In addition, fasting increases gastric ghrelin mRNA expression in mice (Xu et al., 2009) and rats (Toshinai et al., 2001; Kim et al., 2003), whereas gastric ghrelin peptide content is decreased, indicative of increased synthesis and release of the peptide into the circulation by feeding (Toshinai et al., 2001; Kim et al., 2003). Likewise, gastric GOAT expression as well as circulating levels of GOAT protein are increased under conditions of fasting (Gonzalez et al., 2008; Stengel et al., 2010d). Total ghrelin levels are also influenced by fat mass and body weight with an increase in anorexic and cachectic patients and a decrease under conditions of overweight and obesity (Tschöp et al., 2000, 2001b; Cummings et al., 2002). Interestingly, acyl and desacyl ghrelin can be regulated differently as shown by a recent study reporting the release of desacyl ghrelin when the gastric pH is low whereas acyl ghrelin is not affected (Mizutani et al., 2009). Conversely, a rapid decline in fasting circulating levels of acyl ghrelin with less prominent or no changes in desacyl ghrelin was induced in response to various stressors either physical (strenuous physical exercise in humans; Shiiya et al., 2011), immunological (intraperitoneal low dose of lipopolysaccharide (Stengel et al., 2010b) or visceral (abdominal surgery; Stengel et al., 2011b) in rats. This differential regulation of ghrelin and desacyl ghrelin under various physiological conditions is not well understood yet and warrants further characterization.

The regulation of ghrelin secretion by peripheral administration of a large number of gut peptides or transmitters was tested *in vivo* in rats and more recently *in vitro* using ghrelin producing mouse ghrelinoma MGN 3-1 cells as well as in humans (Mundinger et al., 2006; Hosoda and Kangawa, 2008; Zhao et al., 2010; Iwakura et al., 2011). It is well established that somatostatin inhibits ghrelin release in experimental and clinical studies (Shimada et al., 2003; de la Cour et al., 2007; Iwakura et al., 2011). Convergent anatomic and functional evidence indicates a direct action of somatostatin on rat X/A-like and human P/D₁ cells mediated through interaction with the somatostatin receptor subtype 2 (sst₂). This is supported by the expression of sst₂ on ghrelin-positive cells in the rat (Figures 2A,B; Stengel et al., 2011b) and human (Fischer et al., 2008) stomach along with the potent inhibitory effect of intravenous injection of a selective peptide sst₂ agonist (Figures 2C,D; Stengel et al., 2011b). Studies with other gut peptides have yielded inconsistent results that may be related to species differences or experimental conditions. Inhibitory effects were reported for glucagon-like peptide (GLP-1)

in rat and human studies (Lippl et al., 2004; Hagemann et al., 2007; Perez-Tilve et al., 2007) but not in other studies in rats or *in vitro* testing (Mundinger et al., 2006; Hosoda and Kangawa, 2008; Zhao et al., 2010), for CCK-8 in humans (Brennan et al., 2007) but not in rats or *in vitro* (de la Cour et al., 2007; Iwakura et al., 2011), and for insulin in humans and *in vitro* (Saad et al., 2002; Iwakura et al., 2011) but not in rats although a trend was observed (de la Cour et al., 2007). The decrease following administration of bombesin/gastrin releasing peptide in rats (de la Cour et al., 2007) has to be substantiated in further investigations. Other peptides, namely calcitonin, gastrin, gastric inhibitory polypeptide, neurotensin, pancreatic polypeptide, and vasoactive intestinal peptide (VIP) had no effect on ghrelin release both *in vivo* in rats or *in vitro* (de la Cour et al., 2007; Iwakura et al., 2011) while the increase in ghrelin release induced by oxytocin and vasopressin reported *in vitro* (Iwakura et al., 2011) and secretin (de la Cour et al., 2007) as well as endothelin 1 and 3 (de la Cour et al., 2007; Thanthan et al., 2009) *in vivo* still need replication. Among the neurotransmitters increasing ghrelin levels are adrenaline, and noradrenaline

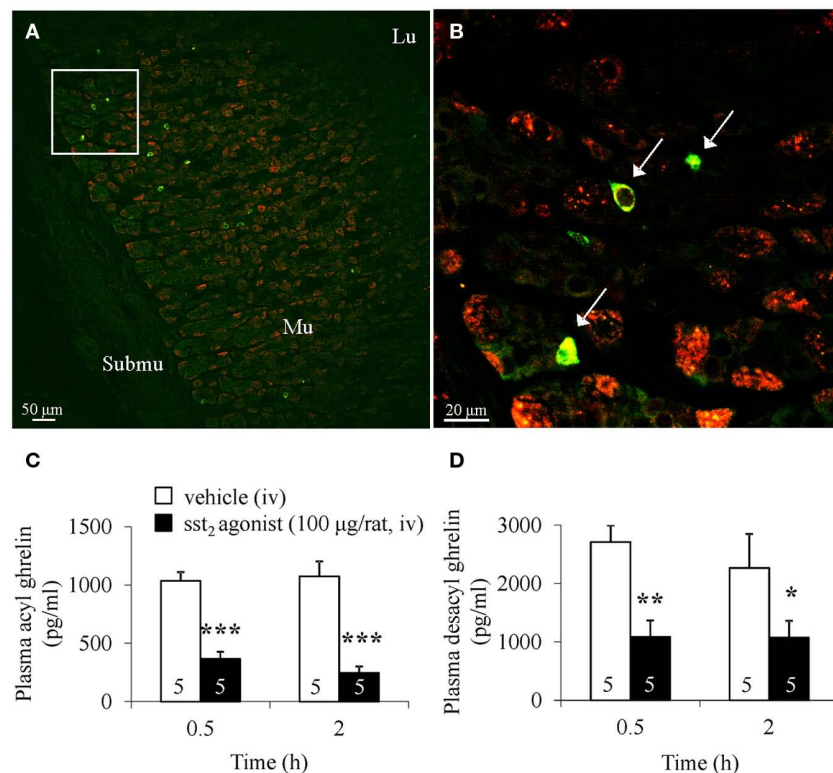


FIGURE 2 | Somatostatin could act directly on gastric X/A-like cells by interaction with the somatostatin receptor subtype 2 (sst₂) localized on these cells. (A,B) Ghrelin-positive X/A-like cells in the gastric oxyntic mucosa express the sst_{2a} receptor in naive rats. Paraffin-embedded gastric corpus sections of *ad libitum* fed rats were processed for immunofluorescent double labeling. Confocal microscopy shows the localization of sst_{2a}-immunopositive cells throughout the gastric oxyntic mucosa [(A), red], whereas ghrelin-positive cells are localized mostly in the middle and the lower part of the glands [(A), green]. The higher magnification (B) shows that ghrelin-positive cells express the sst_{2a} receptor (arrow). Lu, lumen; Mu, mucosa; Submu, submucosa. (C,D) The

sst₂ agonist decreases circulating acyl and desacyl ghrelin levels in overnight-fasted rats implanted with an intrajugular catheter. The sst₂ agonist (100 μg/rat in 200 μl saline containing 0.1% BSA) or vehicle (saline containing 0.1% BSA) was injected intravenously (iv) twice at 0 and 0.5 h. Blood was withdrawn before the second injection at 0.5 and at 2 h and processed for acyl (A) and total ghrelin measurement. Desacyl ghrelin (B) was calculated as the difference of total minus acyl ghrelin for each individual sample. Each bar represents the means ± SEM of number of rats indicated at the bottom of the column. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001 versus vehicle. Reproduced with permission from reference (Stengel et al., 2011b).

in rats and *in vitro* (Mundinger et al., 2006; de la Cour et al., 2007; Iwakura et al., 2011) supporting the contention that the sympathetic nervous system is an important stimulant of ghrelin secretion although other evidence indicates that the neural, unlike the neurohumoral component of the sympathetic nervous system stimulates ghrelin secretion (Mundinger et al., 2006). Less consistent results have been observed for dopamine resulting in ghrelin increase at high concentrations (10^{-4} to 10^{-5}) *in vitro* (Iwakura et al., 2011) but not *in vivo* (de la Cour et al., 2007) while no effect of γ -aminobutyric acid (GABA), serotonin, and histamine have been reported both *in vitro* and *in vivo* (de la Cour et al., 2007; Iwakura et al., 2011). Also activation of vagal cholinergic components of the autonomic nervous system results in the stimulation of ghrelin release in rats (Ao et al., 2006; Stengel et al., 2010a) and in humans in response to sham feeding (Simonian et al., 2005). However, *in vitro* carbachol as well as superfused onto the gastric submucosa had no effect on ghrelin release (de la Cour et al., 2007; Iwakura et al., 2011). Other studies reported a stimulatory effect of cannabinoids (Zbucki et al., 2008) and an inhibitory action of interleukin-1 (Wang et al., 2006) to be further characterized. Prostacyclin induces a reduction of circulating total ghrelin levels that may reflect a direct effect on the prostacyclin receptor (PGI₂) expressed on ghrelin containing cells (Madison et al., 2008). However, in general our knowledge on the regulation of acyl and desacyl ghrelin release at the cellular level is very limited due to the lack of cell isolation techniques of native ghrelin cells. Coexpression of ghrelin and fluorescent dye (Kageyama et al., 2008) or immortalized ghrelin cell lines (Iwakura et al., 2011) will help to advance our understanding on the processing and regulation of ghrelin.

Ghrelin binds to the seven transmembrane domains GRLN-R expressed in the periphery and brain in experimental animals and humans (Howard et al., 1996; Guan et al., 1997; Nakazato et al., 2001; Gnanapavan et al., 2002; Zigman et al., 2006). In peripheral organs, the GRLN-R has been described in the pituitary, on vagal afferents, pancreas, spleen, myocardium, adipose tissue, thyroid, and adrenal gland (Hattori et al., 2001; Gnanapavan et al., 2002; Schellekens et al., 2010). Interestingly, in the brain the GRLN-R is able to form heterodimers with other receptors such as the cannabinoid 1 (CB₁) receptor (Schellekens et al., 2010) and the dopamine receptor subtype 1 (D₁; Jiang et al., 2006) possibly leading to amplified signaling. On the other hand, the GRLN-R rapidly desensitizes after stimulation through endocytosis *via* clathrin-coated pits and shows slow non-dissociation of the ligand/receptor (Camina et al., 2004), mechanisms likely to prevent over-stimulation of the receptor.

BIOLOGICAL ACTIONS OF GHRELIN

The peptide hormone ghrelin exerts pleiotropic biological actions, prominently on the regulation of food intake, gastrointestinal motility, and energy homeostasis. In addition, there is a growing body of evidence that ghrelin is also involved in stress pathways as recently reviewed (Stengel et al., 2011c). Lastly, ghrelin seems to be involved in the modulation of reproductive, cardiovascular, and immune functions. Although additional functions and pharmacological effects have been described for ghrelin (Ueno et al., 2010; Steiger et al., 2011; Ukkola, 2011) this review will focus on those listed above.

OREXIGENIC EFFECT

Ghrelin is the only known peripherally produced and centrally acting hormone that is well established to stimulate food intake following peripheral or brain injection in various animal models (Wren et al., 2000; Tang-Christensen et al., 2004) as well as after peripheral administration in lean and obese humans (Druce et al., 2005). Similar to acyl (octanoyl) ghrelin, *n*-decanoyl ghrelin stimulates food intake in mice (Hiejima et al., 2009). These effects are mediated by interaction with the GRLN-R as conclusively shown by the suppression of ghrelin-induced food intake by GRLN-R antagonists (e.g., JMV 3002, 2959, and 2810; Salome et al., 2009). Further supporting these results GRLN-R knockout mice failed to increase food intake following exogenous administration of ghrelin (Sun et al., 2004; Zigman et al., 2005). Interestingly, mice over-expressing both ghrelin and desacyl ghrelin do not display stimulated but rather decreased food intake (Iwakura et al., 2009) which is not related to the desensitization of receptors. This is suggested to be related to the gastric hypertrophy under these conditions, although the balancing role of desacyl ghrelin counteracting the orexigenic effect of ghrelin could not be ruled out as recently reviewed (Stengel et al., 2010c).

Ghrelin exerts its orexigenic effects *via* direct actions on food regulatory brain nuclei after passage through the blood–brain barrier (Banks et al., 2002; Pan et al., 2006). Moreover, the GRLN-R is expressed on vagal afferents (Date et al., 2002a; Sakata et al., 2003) and therefore a mediation of the food intake stimulatory signals *via* the vagus nerve has been proposed. In line with this assumption, the ghrelin-induced food intake following intravenous injection in rats (4.9 μ g/rat) is abolished after subdiaphragmatic or gastric vagotomy (Date et al., 2002a). However, in contrast with this finding another study reported that after selective subdiaphragmatic vagal deafferentation intraperitoneally injected ghrelin (40 μ g/kg, approximately 12 μ g/rat) still stimulates food intake in rats (Arnold et al., 2006). Whether this merely represents a dose-related effect with recruitment of both vagal and direct central mechanisms with increasing doses of peptides warrants further investigation. Collectively, existing data support the contention that ghrelin is likely to increase food intake *via* both, direct brain action and vagal pathways. Lastly, ghrelin is not only involved in the homeostatic control of food intake but also in the rewarding aspect of food as recently reviewed (Skibicka and Dickson, 2011). This was established by showing that the peripheral injection of ghrelin in mice increases the consumption of saccharin irrespective of the caloric content, an effect that is no longer observed in mice lacking the GRLN-R (Disse et al., 2010).

INTERACTION WITH APPETITE-REGULATING PEPTIDES IN THE HYPOTHALAMUS AND MOLECULAR MECHANISMS

Ghrelin is also produced centrally in the arcuate nucleus of the hypothalamus (Lu et al., 2002) and in neurons adjacent to the third ventricle (Cowley et al., 2003). The arcuate nucleus plays a major role in the regulation of food intake (Schwartz et al., 2000) and neuroanatomical studies showed that ghrelin containing arcuate neurons are connected with agouti-related peptide (AgRP) and neuropeptide Y (NPY) positive neurons (Cowley et al., 2003; Guan et al., 2003), two major central orexigenic peptides (Abizaid and Horvath, 2008). Peripheral administration of

ghrelin selectively activates NPY immunoreactive arcuate neurons in mice (Wang et al., 2002). Likewise, acute and chronic intracerebroventricular (icv) injection of ghrelin activates NPY/AgRP positive neurons and upregulates the expression of NPY and AgRP mRNA (Kamegai et al., 2001). Pharmacological studies showed that ghrelin's orexigenic action is abolished by an anti-NPY or anti-AgRP antibody (Nakazato et al., 2001). Gene deletion experiments further highlight these findings. Mice lacking NPY and AgRP do not respond to peripherally injected ghrelin whereas mice with a single peptide gene knockout still showed an increased food intake giving rise to a compensation of NPY by AgRP and *vice versa* (Chen et al., 2004). Taken together, these data along with the expression of GRLN-R in over 90% of all NPY neurons in the ARC while the GRLN-R is only present in less than 8% of pro-opiomelanocortin (POMC) neurons (Willeesen et al., 1999) show that the orexigenic effect of ghrelin is mediated by central AgRP and NPY signaling. However, while the orexigenic NPY/AgRP neuronal activity is stimulated, the activity of POMC containing neurons is suppressed *via* inhibitory GABAergic inputs. This lowering of activity of POMC promotes ghrelin's orexigenic action by dampening melanocyte stimulating hormone (the peptide product of POMC) and cocaine- and amphetamine-regulated transcript (CART) anorexigenic pathways (Cowley et al., 2003).

The downstream signaling mechanisms in arcuate neurons activated by ghrelin have been recently unraveled and reviewed (Andrews, 2011). They involve AMP-activated protein kinase (AMPK) – carnitine palmitoyltransferase 1 (CPT1) – uncoupling protein 2 (UCP2) pathways that promote mitochondrial efficiency and shield reactive oxygen species in order to maintain an appropriate firing response of NPY. Ghrelin increased mitochondrial respiration in NPY arcuate neurons, an effect that was dependent on UCP2 as shown by the complete absence of these changes in mice lacking the UCP2 gene (Andrews et al., 2008). Ghrelin also increased the number of mitochondria in NPY neurons in wild type but not *Ucp2* knockout mice (Andrews et al., 2008). In mice lacking *Ucp2*, ghrelin increases reactive oxygen species suggesting a buffering system by UCP2 (Andrews et al., 2008). The activation of these NPY neurons as indicated by the increased action potential firing induced by ghrelin was also attenuated in *Ucp2* knockout mice (Andrews et al., 2008). Lastly, the ghrelin-induced food intake was blunted in *Ucp2* knockout mice (Andrews et al., 2008). It has been shown that fasting induces an increased phosphorylation of AMPK in the hypothalamus resulting in decreased hypothalamic levels of malonyl-CoA and increased CPT1 activity (Lopez et al., 2008). This effect on AMPK signaling is mimicked by injected ghrelin (Lopez et al., 2008) pointing toward a physiological mechanism of ghrelin to promote feeding. Further corroborating this hypothesis, central blockade of AMPK signaling by dominant negative forms of AMPK α 1 and α 2 reduced the orexigenic effect of ghrelin (Lopez et al., 2008). Moreover, fasting as well as injection of ghrelin decreases the hypothalamic expression of fatty acid synthase (FAS; Lopez et al., 2008). This fasting induced decrease of FAS mRNA expression is absent in GRLN-R knockout mice (Lopez et al., 2008) indicating a physiological effect of ghrelin. Lastly, inhibition of AMPK signaling blocked the ghrelin-induced reduction of FAS mRNA expression (Lopez et al., 2008) providing insight into the downstream signaling of ghrelin to mediate the

central orexigenic action. The activation of AMPK signaling by ghrelin was still visible in *Ucp2* knockout mice (Andrews et al., 2008) pointing toward an effect upstream of UCP2. On the other hand, inhibition of AMPK signaling reduced food intake in wild type but not *Ucp2* knockout mice indicating that UCP2 is relevant downstream of AMPK (Andrews et al., 2008). Chronic treatment with ghrelin results in greater body weight gain in mice lacking the UCP2 gene compared to wild type littermates due to decreased fat oxidation (Andrews et al., 2010) highlighting the importance of UCP2 for the restriction of fat storage.

EFFECTS ON ENERGY HOMEOSTASIS

In addition to its effects on short term modulation of food intake, ghrelin also affects long term body weight homeostasis. Chronic infusion of ghrelin enhances body weight gain in rodents which is not only due to increased appetite but also to fat storage (Tschöp et al., 2000; Strassburg et al., 2008; Davies et al., 2009) especially in retroperitoneal and inguinal fat tissue (Davies et al., 2009; Sangiao-Alvarellos et al., 2009). On the other hand, mice that over-express desacyl ghrelin have a decreased body weight compared to their wild type littermates (Ariyasu et al., 2005) associated with reduced amounts of perirenal and epididymal fat tissue (Zhang et al., 2008). Since the food intake was not changed in those mice (Ariyasu et al., 2005), these alterations could reflect a direct effect of desacyl ghrelin on fat storage. However, in humans both acylated and desacyl ghrelin have been reported to stimulate lipid accumulation in visceral adipocytes (Rodriguez et al., 2009). Besides the effect on increased storage, ghrelin also increases fat depots by reducing lipid mobilization as indicated by an increased respiratory exchange ratio (Davies et al., 2009). Conversely genetically modified mice lacking both ghrelin and the GRLN-R display increased energy expenditure resulting in a decrease of body weight (Pfluger et al., 2008). Interestingly, mice lacking either ghrelin (Sun et al., 2003; Pfluger et al., 2008) or the GRLN-R (Pfluger et al., 2008) genes do not show these alterations. However, GRLN-R knockout mice fed a high fat diet (a.k.a. western type diet) gain less body weight than their wild type littermates (Zigman et al., 2005). Additional support for a role of ghrelin in energy homeostasis comes from ghrelin knockout mice fed a high fat diet that show a reduced respiratory quotient indicating increased fat utilization (Wortley et al., 2004). When ghrelin was infused chronically icv glucose utilization was increased in brown adipose tissue (Theander-Carrillo et al., 2006). Ghrelin increased the mRNA expression of FAS, acetyl-CoA carboxylase alpha, stearoyl-CoA desaturase-1, and lipoprotein lipase (Theander-Carrillo et al., 2006), all enzymes involved in promotion of fat storage. On the other hand, the mRNA expression of the major enzyme of fat oxidation, carnitine palmitoyltransferase-1alpha was reduced. Supporting a physiological relevance of these findings, ghrelin deficient mice showed an opposite expression pattern of these enzymes (Theander-Carrillo et al., 2006). Interestingly, mice lacking the β 1-, β 2-, and β 3-adrenoceptor treated icv with ghrelin did not gain body weight and did not show an up-regulated mRNA expression of, e.g., FAS as observed in wild type mice (Theander-Carrillo et al., 2006) indicating a crucial involvement of the sympathetic nervous system in the homeostatic effects of ghrelin. In summary, ghrelin is involved in the maintenance of energy homeostasis and promotes fat storage.

The role of GOAT in energy homeostasis was investigated using mice lacking *Mboat4* or over-expressing human ghrelin and GOAT (Kirchner et al., 2009). Mice lacking *Mboat4* did not show changes in body weight when fed a standard rodent diet but developed a reduced body weight when fed a high fat diet compared to their wild type littermates (Kirchner et al., 2009). Furthermore, adding specifically medium-chain triglycerides to the diet results in lowering of fat mass and body weight of *Mboat4* knockout mice (Kirchner et al., 2009) leading to the hypothesis that GOAT is acting as a lipid sensor.

EFFECTS ON GASTROINTESTINAL MOTILITY

Several food intake regulatory peptides also influence gastrointestinal motility and therefore alteration of this function was early on suspected for ghrelin. In addition, the variation of circulating ghrelin levels is closely associated with the occurrence of gastric migrating motor complexes (Ariga et al., 2007). When injected intravenously, ghrelin increases antral motility in rats (Masuda et al., 2000; Fujino et al., 2003), dogs (Yin and Chen, 2006), and humans (Tack et al., 2006). Conversely, GRLN antagonists abolish gastric phase III-like contractions in mice (Zheng et al., 2009a) and rats (Ariga et al., 2007; Taniguchi et al., 2008a). In addition, ghrelin stimulates the antropyloric coordination when injected intraperitoneally (Ariga et al., 2008). The increased gastric propagative contractile activity could underlie the ghrelin-induced acceleration of gastric emptying of liquid and solid food in rats (Trudel et al., 2002; Fukuda et al., 2004; Depoortere et al., 2005; Wang et al., 2006; Tumer et al., 2008), mice (Asakawa et al., 2001b; Dornonville de la Cour et al., 2004; Kitazawa et al., 2005), and humans (Levin et al., 2006). Therefore, ghrelin is also proposed to act as a gastropromotor under conditions of reduced gastric motility such as diabetic gastroparesis or after abdominal surgery known to induce postoperative gastric ileus (Camilleri et al., 2009; Sallam and Chen, 2010; Stengel and Taché, 2011). Interestingly, only pharmacologically high doses of ghrelin are able to provoke a gastropromotor effect in humans with neurogenic, diabetic, or idiopathic gastroparesis (Murray et al., 2005; Tack et al., 2005; Binn et al., 2006), whereas lower doses that have an effect on GH release do not modulate gastric emptying (Cremonini et al., 2006).

In addition to these effects in the stomach, intravenous infusion of ghrelin also increases small intestinal interdigestive contractions (Edholm et al., 2004; Taniguchi et al., 2008b) resulting in an acceleration of small intestinal transit in rats (Trudel et al., 2002; Fukuda et al., 2004). Likewise, also colonic transit is increased by intravenous (Shimizu et al., 2006), intrathecal (Shimizu et al., 2006) as well as third intracerebroventricular injection (Tebbe et al., 2005) of ghrelin or ghrelin agonists.

EFFECTS ON REPRODUCTIVE FUNCTIONS

Ghrelin has only recently emerged as a possible signal to participate in the regulation of reproductive physiology by both hormonal actions at different levels of the reproductive system as well as by direct gonadal actions in males and females as recently reviewed in detail (Muccioli et al., 2011). Supporting a local action, ghrelin is expressed in the rat testis (Barreiro et al., 2002) and similar to rodent models, ghrelin RNA expression was also detected in human testis (Gnanapavan et al., 2002; Gaytan et al., 2004) with

a cellular distribution pattern in Leydig cells (Gaytan et al., 2004) and Sertoli cells (Barreiro et al., 2003). Along with the ligand, the GRLN-R has been localized in rat and human testis on different cell types, namely the Leydig cells, Sertoli cells, and probably germ cells (Barreiro et al., 2003; Gaytan et al., 2004). Likewise, in several female animal species and humans, the expression of ghrelin has been documented in ovary, hilus interstitial cells and young and mature corpora lutea and that of GRLN-R protein in oocytes, somatic follicular cells, hilus interstitial cells, and luteal cells (Muccioli et al., 2011).

In keeping with high demand in energy of the reproductive axis, ghrelin acting at central and peripheral levels is largely inhibitory. When ghrelin is injected centrally in rats, the peptide suppresses luteinizing hormone (LH) secretion in ovariectomized female rats (Furuta et al., 2001) as well as cyclic female rats (Fernandez-Fernandez et al., 2006). Similarly, gonadotropin releasing hormone (GnRH) is significantly inhibited by ghrelin (Fernandez-Fernandez et al., 2006). This inhibition could be, at least in part, due to a suppression of *Kiss1* gene expression (Forbes et al., 2009). However, *in vitro* studies reported a stimulatory effect of ghrelin on LH and follicle stimulating hormone (FSH) release from pituitary tissue (Fernandez-Fernandez et al., 2006) indicating a differential mode of action when injected centrally *versus* applied locally on the pituitary gland. Consistent with its inhibitory effects on LH and FSH release, ghrelin delayed balano-preputial separation (Zigman and Elmquist, 2003; Fernandez-Fernandez et al., 2005b; Martini et al., 2006) and vaginal opening (Fernandez-Fernandez et al., 2005a) as an indicator of puberty in male and female rats, respectively.

EFFECTS ON CARDIOVASCULAR FUNCTIONS

Ghrelin and the GRLN-R are also expressed in cardiomyocytes with decreased hormone and increased receptor expression in patients with congestive heart failure (Beiras-Fernandez et al., 2010) which supports the effects of ghrelin on cardiovascular functions. In cultured H9c2 cardiomyocytes, ghrelin and the ghrelin mimetic hexarelin increase proliferation as indicated by increased thymidine incorporation, an effect that is likely to be mediated by a receptor different than the GRLN-R due to binding of ghrelin and hexarelin also in the absence of the GRLN-R (Pettersson et al., 2002). In addition, ghrelin also exerts antiapoptotic effects on mouse and rat cardiomyocytes by acting on MAPK and PI3K/Akt pathways, an effect also mimicked by desacyl ghrelin (Baldanzi et al., 2002; Lear et al., 2010). Moreover, hexarelin reduces apoptosis of rat cardiomyocytes by blocking caspase-3 activity, reducing the expression of the proapoptotic Bax protein and increasing expression levels of the antiapoptotic Bcl-2 (Pang et al., 2004).

Besides the effects on proliferation and controlled cell death, ghrelin also affects blood pressure and cardiac output. The GH secretagogue induced an increase in cardiac output in rats (Nagaya et al., 2001b) and humans (Bisi et al., 1999) which may be a direct effect (Enomoto et al., 2003) but also be secondary to the vasodilatation or release of GH (Tajima et al., 1999). Ghrelin decreases blood pressure (Nagaya et al., 2001a) likely through activating endothelial nitric oxide synthase (eNOS; Shimizu et al., 2003) but also nitric oxide independent mechanisms (Okumura et al., 2002).

In addition, ghrelin also exerts a direct inhibitory effect on sympathetic nervous activity when microinjected into the nucleus of the solitary tract in rats, a major autonomic brain nucleus in the brain-stem (Lin et al., 2004). Conversely, reduction of ghrelin signaling by peripheral administration of the GRLN-R antagonist, [D-Lys-3]-GHRP-6 increases heart rate and mean arterial pressure, an effect blocked by inhibition of α - and β_1 -adrenoreceptors (Vlasova et al., 2009).

These effects mentioned above may have clinical significance mainly under conditions of cardiac ischemia. Hexarelin (Locatelli et al., 1999) and ghrelin (Frascarelli et al., 2003) protect against cardiac damage as shown by a decrease in infarction size post ischemia. In line with these findings, the injection of ghrelin during reperfusion after an ischemic period in rats exerts beneficial effects on heart functions as indicated by increased left ventricular contraction and improved left ventricular systolic pressure and coronary flow (Chang et al., 2004). Ghrelin may also induce beneficial effects in patients with congestive heart failure as suggested by a pilot study reporting that these subjects treated with ghrelin intravenously over a period of 3 weeks increased left ventricular ejection fraction associated with lower left ventricular end-systolic volume (Nagaya et al., 2004), an interesting finding to be followed up in larger cohorts of patients. Based on growing evidence, the administration of ghrelin might become a unique new therapy for cardiovascular diseases (Kishimoto et al., 2011).

EFFECTS ON STRESS AND COPING FUNCTIONS

Recent studies investigated the role of ghrelin in the stress response and a possible involvement in coping functions. In a model of mice maintained under conditions of caloric restriction, circulating acyl ghrelin levels increased, and animals showed an anxiolytic and antidepressant behavior (Lutter et al., 2008). These types of behavior were mimicked by exogenous administration of ghrelin and not observed in mice lacking the GRLN-R (Lutter et al., 2008). In a different animal model of chronic defeat stress, despite the increase of blood ghrelin levels mice display depression-like symptoms such as reduced social interaction (Lutter et al., 2008). These behavioral alterations are more pronounced in mice lacking the GRLN-R (Lutter et al., 2008) pointing toward a stress coping effect of ghrelin. In line with these findings, circulating levels of ghrelin are increased after restraint stress in rats (Zheng et al., 2009b). Moreover, Wistar Kyoto rats, a rat strain displaying more anxiety than Sprague Dawley rats, have twofold lower fasting ghrelin levels compared to Sprague Dawley rats (Florentzon et al., 2009). Lastly, a study in human subjects reported that intravenous administration of ghrelin reduced the mental stress-induced rise in blood pressure and sympathetic nerve activity (Lambert et al., 2011).

Contrasting with the body of evidence described above, another study reported that peripheral or direct brain injection of ghrelin increases anxiety which is blocked by a non-selective corticotropin releasing factor (CRF) receptor subtypes 1 and 2 antagonist, α -helical CRF₉₋₄₁ (Asakawa et al., 2001a). In addition, ghrelin stimulates CRF release from hypothalamic cells *in vitro* (Kageyama et al., 2011) leading to the hypothesis that ghrelin may increase CRF signaling pathways and thereby some biological components linked with the stress response. In summary, the role of ghrelin

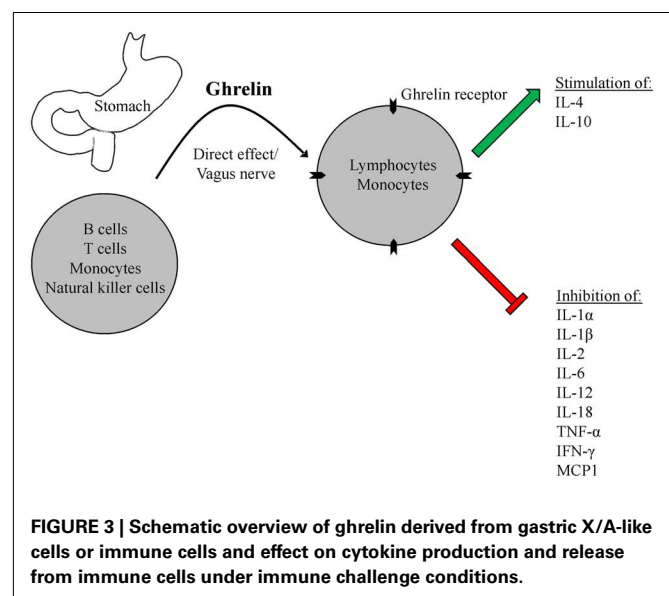
in response to stress and possible coping modulatory properties remain to be further characterized and may differ in states of acute *versus* chronic stress.

EFFECTS ON IMMUNE FUNCTIONS

Expression and effect on cytokine production under acute conditions

Ghrelin, like the expression of several other gut peptides is found in immune cells encompassing B and T cells as well as monocytes and natural killer cells (Hattori, 2009; **Figure 3**). In addition, also the GRLN-R is expressed on rodent immune cells (Gnanapavan et al., 2002) and has subsequently also been detected on human monocytes and T cells (Dixit et al., 2004; **Figure 3**).

Ghrelin and ghrelin agonists have an immunomodulatory protective effect under conditions of acute endotoxemia resulting in reduced tissue infiltration by immune cells (Chen et al., 2008; Li et al., 2010) and decreased mortality (Chang et al., 2003b). This could be mediated directly *via* the interaction with immune cells since ghrelin reduces the mRNA and protein production of the proinflammatory cytokines, interleukin-1 α (IL-1 α), IL-1 β , IL-6, and tumor necrosis factor α (TNF- α) after an immune challenge, an effect not observed with desacyl ghrelin (Dixit et al., 2004; **Figure 3**). Ghrelin affects both the Th1 and the Th2 pathways as shown by the suppression of IL-2 and interferon- γ and IL-4 and IL-10 respectively in mice (Xia et al., 2004). Conversely, when ghrelin expression is knocked down in T cells by silencing RNA, levels of proinflammatory cytokines such as interferon- γ and IL-17 were increased giving rise to a physiological role of ghrelin in regulating the inflammatory response (Dixit et al., 2009). One important regulatory pathway targeted seems to be the high mobility group box 1 (HMGB1), a DNA-binding factor acting as late inflammatory factor crucial for progression of sepsis (Chorny et al., 2008) and activating peroxisome proliferator-activated receptor gamma (PPAR gamma; Cheng et al., 2009), whose inhibition by ghrelin resulted in blunted circulating cytokine levels (Chorny et al., 2008). Similar to the orexigenic effect, the vagus nerve seems to



play a role in the mediation of ghrelin's effects on immune actions as after vagotomy IL-6 and TNF- α levels were not blocked by ghrelin under immune challenge conditions (Wu et al., 2007a). An overview of studies reporting the effects of ghrelin treatment on cytokine levels is given in **Table 1**.

The use of the endotoxin LPS which is part of the membrane of Gram-negative bacteria is a well established model of acute immune stress. Under these conditions ghrelin reduces NF κ B activity and lowers the circulating levels of TNF- α , monocyte chemotactic protein-1 (MCP-1), and IL-8 (Li et al., 2004). This effect was also reproduced in human cells where ghrelin blocked the expression of proinflammatory cytokines in human monocytes following an LPS challenge (Dixit et al., 2004). Several studies investigated the regulation of ghrelin under these conditions and reported a decrease of circulating ghrelin levels following endotoxin challenge or cytokine injection (Basa et al., 2003; Hataya et al., 2003; Wang et al., 2006; Endo et al., 2007; Vila et al., 2007). Similarly, after cecal ligation and puncture in rats, the circulating levels of ghrelin were reduced as well as the GRLN-R mRNA expression in the intestine, aorta, and heart (Wu et al., 2005) indicating not only a regulatory but also adaptive role of the ghrelin signaling system under these conditions.

Effects on cytokine production under subacute and chronic conditions

Contrasting to the acute regulation, under chronic conditions, circulating ghrelin levels were reported to be increased during postoperative sepsis (Maruna et al., 2005) as well as in a mouse model of trinitrobenzene sulfate (TNBS)-induced acute colitis

(Gonzalez-Rey et al., 2006). In this colitis model, ghrelin injected intraperitoneally improves clinical signs of illness along with histological signs of colitis by reducing the mRNA and protein expression of inflammatory cytokines such as TNF- α , IFN- γ , IL-1 α , IL-1 β , IL-6, IL-12, IL-15, IL-17, and IL-18 and increasing the colonic levels of the anti-inflammatory cytokine IL-10 (Gonzalez-Rey et al., 2006).

In a rat model of cardiac ischemia chronic ghrelin treatment over a period of 4 weeks inhibited myocardial remodeling and thereby improved cardiac functions (Huang et al., 2009). Similarly, subcutaneous ghrelin treatment over a period of 5 weeks reduced the clinical severity of experimental allergic encephalomyelitis, a mouse model of multiple sclerosis which was associated with the reduction of proinflammatory cytokines including TNF- α , IL-1 β , and IL-6 probably derived from microglia (Theil et al., 2009).

Lastly, the role of ghrelin signaling was also investigated in the context of rheumatoid arthritis. Ghrelin mRNA and peptide expression has been detected in mouse, rat, and human chondrocytes (Caminos et al., 2005). In addition, GOAT mRNA expression was detected in cultured murine and human immortalized chondrocytes and found to be decreased by LPS (Gomez et al., 2009). In a rat model of adjuvant-induced arthritis and in patients with rheumatoid arthritis circulating ghrelin levels were reduced (Otero et al., 2004). Exogenous administration of ghrelin was shown to attenuate arthritis in the rodent model associated with a decreased production of TNF- α and IFN- γ whereas expression of the anti-inflammatory cytokine IL-10 was increased (Granado et al., 2005a,b). These studies may be indicative of a potential use of ghrelin or ghrelin agonists in the treatment of rheumatoid arthritis.

Table 1 | Effects of ghrelin treatment on cytokine levels in animal models of inflammation.

Species	Condition	Effect of ghrelin	Reference
Mouse	LPS 10 μ g/mouse	Suppression of serum TNF- α , IL-1 β , IL-6	Dixit et al. (2004)
Mouse	LPS 400 μ g/mouse, ip	Suppression of serum TNF- α , IL-1 β , IL-6, IL-12	Chorny et al. (2008)
Mouse	LPS 3.5 mg/kg, ip	Suppression of serum and kidney tissue TNF- α , IL-1 β , IL-6	Wang et al. (2009)
Mouse	TNBS-induced colitis	Suppression of colonic mucosal and serum TNF- α , IL-1 β , IL-6	Gonzalez-Rey et al. (2006)
Mouse	Experimental allergic encephalomyelitis	Suppression of spinal cord tissue TNF- α , IL-1 β , IL-6	Theil et al. (2009)
Rat	LPS 10 mg/kg, iv	Suppression of serum TNF- α , IL-8, MCP-1	Li et al. (2004)
Rat	LPS, it	Suppression of bronchial alveolar lavage fluid TNF- α , IL-1 β	Chen et al. (2008)
Rat	Cecal ligation and puncture	Suppression of peritoneal fluid and serum TNF- α , IL-6	Wu et al. (2007a,b)
Rat	Cecal ligation and puncture	Suppression of liver MKP1	Jacob et al. (2010)
Rat	Acetaminophen induced liver injury	Suppression of liver TNF- α	Golestan Jahromi et al. (2010)
Rat	Bile duct ligation	Suppression of serum TNF- α , IL-1 β , IL-6	Iseri et al. (2008)
Rat	Pancreatitis by sodium taurocholate injection	Suppression of serum TNF- α , IL-1 β , IL-6	Zhou and Xue (2009)
Rat	Intestinal ischemia	Suppression of serum TNF- α , IL-6	Wu et al. (2008)
Rat	Cardiac ischemia	Cardiac neighboring tissue levels of TNF- α , IL-1 β	Yuan et al. (2009)
Rat	Cardiac ischemia	Cardiac neighboring tissue levels of TNF- α , IL-1 β mRNA, MMP2, MMP9	Huang et al. (2009)
Rat	Sciatic nerve ligation	Suppression of spinal cord tissue TNF- α , IL-1 β	Guneli et al. (2010)
Rat	Subarachnoid hemorrhage	Suppression of serum TNF- α , IL-1 β	Ersahin et al. (2010)
Rat	Cerebral ischemia	Suppression of cerebral tissue TNF- α , IL-6 mRNA	Cheyuo et al. (2010)
Rat	Chronic renal failure by nephrectomy	Suppression of serum TNF- α , IL-1 β , IL-6	Deboer et al. (2008)

ip, Intraperitoneally; it, intrathecally; iv, intravenously; MKP1, mitogen activated protein kinase phosphatase-1; MMP, matrix metalloproteinase.

ALTERATIONS OF CIRCULATING GHRELIN BY STRESS OR IMMUNE CHALLENGES

A number of studies have investigated the impact of various challenges on circulating levels of ghrelin and desacyl ghrelin in experimental animals and humans as recently reviewed (Stengel et al., 2011c).

PSYCHOLOGICAL STRESSORS

In a model of water avoidance stress, this psychological stressor applied acutely (for 90 min) or continuously (over a period of 5 days) increases circulating acyl (Ochi et al., 2008) and total (Kristensson et al., 2006; Ochi et al., 2008) ghrelin levels in rats. This was associated with an increased expression of gastric ghrelin mRNA (Zheng et al., 2009b). Such changes in ghrelin levels were shown to play a physiological role in the restoration of gastric emptying inhibited acutely by such a stress exposure (Ochi et al., 2008). Similarly, in a mouse model of induced depression by daily social defeat associated with exposure to aggressive CD1 mice over a period of 10 days circulating ghrelin levels were increased after the stress on day 11 and remained elevated for a period of 4 weeks (Lutter et al., 2008). This increase contributes to the blunting of deleterious effects of chronic stress such as reduced social interaction and food intake (Lutter et al., 2008). Another model of chronic unpredictable stress consisting of heterotypic stressors such as noise, open field, aggressive male, novel aversive environment, predator scent, and restraint over a period of 14 days results in an increase of blood acyl ghrelin levels in mice (Patterson et al., 2010). These effects are not restricted to animal models as the Trier Social Stress Test, a well described stress test for humans where participants have to give a speech in front of an expert committee being videotaped, leads to an increase of circulating total ghrelin levels (Rouach et al., 2007; Raspopow et al., 2010). In summary, various psychological stressors increase circulating ghrelin levels not only in animals but also in humans. This increase could play a role in the defense against depressive-like symptoms (Lutter et al., 2008) and stress-induced eating and food-reward behavior (Chuang et al., 2011) under conditions of chronic stress as recently hypothesized.

ENDOTOXIN

Injection of LPS at a low dose (100 µg/kg) mimics symptoms of an acute infection including reduced appetite and increased body temperature (Langhans, 1996, 2000; Wang et al., 2006). At the same time, LPS decreases fasting levels of circulating total ghrelin in rats (Basa et al., 2003; Wang et al., 2006; Stengel et al., 2010b). This decrease was rapid in onset and long lasting and levels were completely restored at 24 h post injection (Wang et al., 2006; Stengel et al., 2010b). Exogenous administration of ghrelin under these conditions restored both gastric emptying and food intake which may be indicative that alterations in circulating ghrelin could be part of the underlying mechanisms associated with alterations of ingestive and gut function in response to bacterial infections. Also, studies in human subjects reported a decrease of circulating ghrelin at 5 h post injection (Vila et al., 2007). However, this was preceded by a rapid increase of ghrelin at 2 h post injection (Vila et al., 2007). In addition, under conditions of chronic infection with the Gram-negative bacterium, *Helicobacter pylori*, circulating ghrelin levels are also decreased when compared to *H. pylori* negative individuals (Jeffery et al., 2011) and eradication of

the bacteria was reported to restore ghrelin levels and to improve appetite and increase body weight (Jeffery et al., 2011). In contrast to these states of mild inflammation, when assessed under conditions of septic shock, circulating ghrelin levels were increased in fasted dogs after injection of high dose endotoxin (1 mg/kg; Yilmaz et al., 2008) and in fasted rats after cecal ligation and perforation (Chang et al., 2003a) that was associated with reduced mean arterial blood pressure and blood glucose levels (Chang et al., 2003a). Likewise, in humans ghrelin levels increased during the first days of sepsis (Maruna et al., 2005). Whether this increase is merely due to reduced ghrelin clearance observed under those conditions (Wu et al., 2003) or also reflects increased production, activation, and release warrants further investigation.

ABDOMINAL SURGERY

Abdominal surgery consisting of laparotomy and cecal palpation is a well established model for visceral stress which induces a rapid and long-lasting decline in fasted plasma acyl and desacyl ghrelin levels observed at 0.5, 2, and 5 h post surgery (Stengel et al., 2010a, 2011b). Ghrelin levels were partly recovered at 7 h and fully restored at 24 h post surgery (Stengel et al., 2011b). This decrease was accompanied by a delay of gastric emptying and a reduction of food intake (Stengel et al., 2011a). Interestingly, in humans an increase of total ghrelin was observed at 24 h post surgery compared to preoperative levels (Maruna et al., 2008), a difference that remains to be further investigated.

SUMMARY

Ghrelin as new gastric hormone has attracted much attention early on due to its stimulatory effects on food intake observed across many species. Thereafter, fields of ghrelin investigation expanded to encompass integrated actions of the orexigenic effect with that on energy homeostasis and gastrointestinal motility. More recently, our knowledge is increasing on the modulatory actions of ghrelin on cardiovascular (antiapoptotic effects, protection against cardiac damage, increase of cardiac output) and reproductive functions (largely inhibitory) along with inflammation (largely suppressing the production of cytokines). In addition, ghrelin, can restore various functions, e.g., gastric emptying affected under conditions of inflammation and stress responses (increases coping, blunts the effects of chronic stress). Although several important questions were answered recently, highly relevant cellular and molecular mechanisms remain to be investigated and clearly defined. These include the receptor expression pattern of the gastric X/A-like cell to characterize the possible direct interactions with other transmitters modulating the expression and release of ghrelin under different conditions. The subcellular mechanisms of ghrelin expression, processing, and release leading to the altered circulating levels of the acyl and non-acylated form of the peptide hormone need to be characterized. Genetic approaches such as labeling with fluorescent dye followed by isolation of the X/A-like cells for microarray as well as *in vitro* analyses will be helpful to approach these goals.

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